Inhibition of Calpain by A-705253 Prevents NMDA-Induced Neurodegeneration in Hippocampal Slice Cultures and in the N. Basalis magnocellularis of Rats

Moeller, Achim H.; Nimmrich, Volker; Szabo, Robert; Nyakas, Csaba; Schoemaker, Hans; Gross, Gerhard; Luiten, Paul

Published in:
Alzheimer & Dementia

DOI:
10.1016/j.jalz.2008.05.1528

Publication date:
2008

APP5 peptide and P165 could promote the proliferation of cultured hippocampal neural stem cells derived from rat embryo, increase the amount of precursor cells of hippocampal dentate gyrus in adult rat brain. However, these two peptides could not affect the differationation of the above precursor cells in vivo or in vitro. The mechanism is probably related with the PI3'K transduction pathway.

P2-448 PRECLINICAL EVALUATION OF CDD-0102, A SELECTIVE M_1 AGONIST WITH POTENTIAL UTILITY IN ALZHEIMER'S DISEASE

William S. Messer[1,2], Karen Steinmetz[3], Toufan Parman[3], Paul Catz[3], Carol Green[3], Debasis Ghosh[2], Wayne Hoss[1,2], Edward J. McGuire[2], *[1]The University of Toledo, Toledo, OH, USA; [2]Cognitive Pharmaceuticals Ltd., Toledo, OH, USA; [3]SRI International, Menlo Park, CA, USA. Contact e-mail: wmesser@utoledo.edu*

Background: Agonists that selectively activate M_1 muscarinic receptors might be useful in treating memory and cognitive deficits, while preventing the formation of amyloid plaques and neurofibrillary tangles associated with Alzheimer's disease. 5-(3-Ethyl-1,2,4-oxadiazol-5-yl)-1,4,5,6-tetrahydropyrimidine trifluoroacetic acid (CDD-0102J) displays functional selectivity for M_1 receptors and enhances memory function in animals with cholinergic deficits. The hydrochloride salt (CDD-0102A) also promotes alpha-secretase activity and reverses the apoptotic effects of Abeta in differentiated PC12 cells. **Methods:** CDD-0102A was evaluated in a series of studies to determine pharmacokinetic parameters, drug metabolism and toxicity. **Results:** Preclinical toxicology studies indicate that CDD-0102A is negative in bacterial mutagenicity, mammalian cell clastogenicity, and mouse micronucleus assays. Moreover, CDD-0102A does not inhibit the HEK-hERG channel current. In a 28-day repeat-dose toxicity study with CDD-0102A in male and female rats, excessive cholinergic stimulation was apparent at high doses yet no overt toxicities were observed. Dogs were more sensitive to CDD-0102A in a comparable study with dose-dependent increased frequency and severity of cholinergic symptoms, although no significant effects on cardiovascular and pulmonary safety parameters were observed. Studies in rats yielded linear pharmacokinetics with dose proportional increases in C_{max} and AUC and an estimated $t_{1/2}$ of 3-5 hr. Comparative drug metabolism studies indicated significant metabolism only in rabbit S9 preparations, with limited metabolism in liver microsomal and S9 preparations from mice, rats, dogs, monkeys and humans. Three potential metabolites were identified from rabbit liver microsomes. **Conclusions:** The preclinical data are being used in planning Phase I clinical studies of CDD-0102A in healthy adult volunteers. Taken together, the data suggest that CDD-0102A may be useful in treating the symptoms and some of the underlying causes of Alzheimer's disease.

P2-449 INHIBITION OF CALPAIN BY A-705253 PREVENTS NMDA-INDUCED NEURODEGENERATION IN HIPPOCAMPAL SLICE CULTURES AND IN THE N. BASALIS MAGNOCELLULARIS OF RATS

Achim H. Moeller[1], Volker Nimmrich[1], Robert Szabo[2], Csaba Nyakas[2], Hans Schoemaker[1], Gerhard Gross[1], Paul Luiten[2], *[1]Abbott, Neuroscience Research, Ludwigshafen, Germany; [2]Department of Molecular Neurobiology, University of Groningen, Haren, Netherlands. Contact e-mail: achim.moeller@abbott.com*

Background: NMDA receptor-mediated excitotoxicity is thought to underlie a variety of neurological disorders. It has recently been shown that calpain activation is involved in NMDA receptor-related excitotoxicity cascades. The aim of the present study was to examine the neuroprotective effects of the novel calpain inhibitor A-705253 (water-soluble, low molecular weight compound; Ki = 27 nM) in hippocampal slice cultures and in vivo, using excitotoxic lesions of the nucleus basalis magnocellularis (NBM) in rat. **Methods:** For analysis of in vitro neurodegeneration we used the model of NMDA-induced spectrin cleavage in hippocampal slice cultures. Various concentrations of A-705253 were applied before NMDA application, and spectrin degradation products were assessed by Western blot analysis. In vivo neuroprotective effects were investigated by measuring functional and morphological deficiencies induced by NMDA-induced lesions of cholinergic neurons in the nucleus basalis magnocellularis (NBM). NMDA was injected unilaterally into the NBM one hour after application of either A-705253 or the respective vehicles. Behavioral effects were recorded 4-6 days after the insult followed by measurement of the density of cholinergic fibers in the parietal cortex and microglial reaction at the lesion site. **Results:** Application of A-705253 significantly prevented NMDA-induced spectrin degradation in slice cultures with an IC_{50} of 100 nM. In vivo, NMDA lesions markedly decreased performance in novel object recognition experiments. The NMDA-lesioned group treated with MK-801 performed at levels comparable to those of the sham-lesioned control. Treatment with A-705253 dose-dependently prevented the deficit. A-705253 (10 mg/kg) completely prevented the effect of the lesion ($p<0.005$). Open field activity was virtually not affected by either lesion or treatment, indicating that lesions did not produce unspecific behavioral effects. Subsequent analysis of choline acetyltransferase in cortex revealed that application of A-705253 dose-dependently prevented neurodegeneration of cholinergic fibers ($p<0.01$ for 3 and 10 mg/kg). The 10 mg/kg dose also significantly prevented the accompanying gliosis, as determined by immunohistochemical analysis of microglial activation. **Conclusions:** We conclude that inhibition of calpain represents a valuable strategy for the prevention of excitotoxicity-induced neurodegeneration and may thus be a novel approach for the treatment of a variety of neurodegenerative disorders.

P2-450 CEREBROLYSIN STIMULATES NEUROGENESIS IN THE DENTATE GYRUS: A BASIS FOR NEURONAL REPLACEMENT THERAPY IN ALZHEIMER'S DISEASE?

Philipp Novak[1], Edward Rockenstein[2], Yoshitaka Tatebayashi[3], Herbert Moessler[1], Khalid Iqbal[4], Eliezer Masliah[2], *[1]Ebewe Pharma, Unterach, Austria; [2]Department of Neurosciences, University of California, San Diego, San Diego, CA, USA; [3]Laboratory for AD, Brain Science Institute, Saitama, Japan; [4]Department of Neurochemistry, New York State Institute for Basic Research in Developmental Disabilities, New York, NY, USA. Contact e-mail: philipp.novak@ebewe.com*

Background: Cerebrolysin is a peptide preparation acting like endogenous neurotrophic factors and has been shown to decrease amyloid burden by regulating APP maturation and transport. Since neurodegenerative alterations in AD might also be related to changes in neurogenesis, these studies aimed to investigate the ability of Cerebrolysin to stimulate neurogenesis in the hippocampus. **Methods:** Transgenic mThy1-hAPP751 (n=24), non-transgenic (n=12) mice and wildtype Wistar rats (n=24) were loaded with BrdU and treated with Cerebrolysin for up to 3 months. Markers for new born cells (BrdU), proliferation (PCNA), migrating neuroblasts (DCX), neuronal phenotype (NeuN) and apoptosis (TUNEL) were analysed in the granular/subgranular layers of the hippocampus. **Results:** The vehicle-treated APP tg mice showed decreased numbers of BrdU+ and DCX+ neural stem cells in the dentate gyrus compared to non-tg controls. APP tg mice treated with Cerebrolysin resulted in a significant increase of BrdU+ cells, DCX+ neuroblasts and a decrease in TUNEL+ neural stem cells compared to vehicle treated APP tg mice. Cerebrolysin did neither change the number of PCNA+ proliferating neural stem cells nor the ratio of BrdU+ cells converting to neurons and astroglia. A significant increase in the number of BrdU positive neurons was also observed in rat hippocampi. Interestingly, these neurons were found more frequently as pairs in Cerebrolysin treated rats than in controls suggesting that Cerebrolysin rescues one of the daughter cells. Furthermore, this increase in number of neurons correlated with improved cognitive performance. **Conclusions:** These findings have shown that Cerebrolysin promotes neurogenesis by protecting neuronal stem cells and decreasing the rate of apoptosis. Together with a reduction of amyloid production, neurogenic effects of Cerebrolysin might contribute to the alleviation of the synaptic and cognitive deficits in patients with AD.